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Use of Gas Chromatography to Identify Geographical Origin of Some Spices

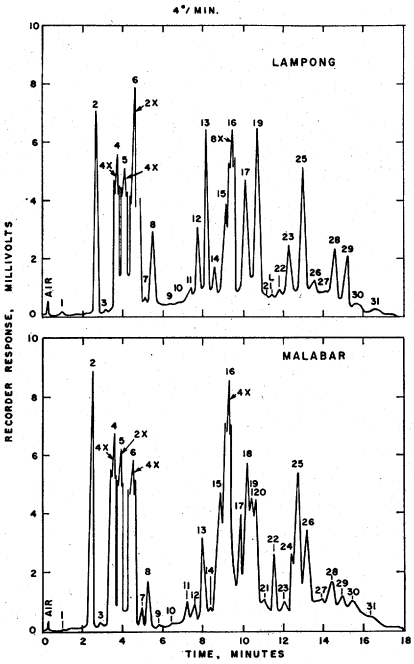

Fig. 1. Linear-temperature-programmed gas chromatograms of steam-volatile oil from two black peppers

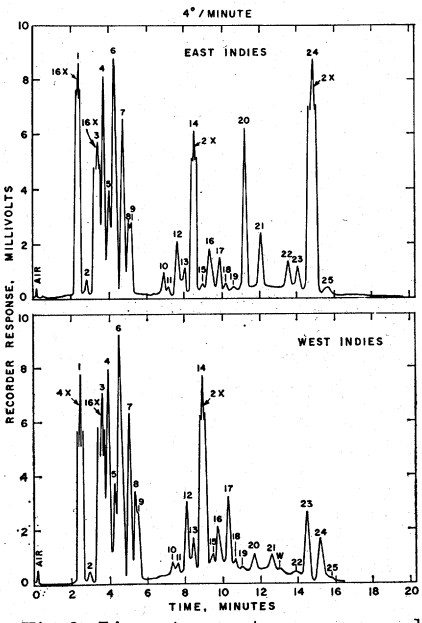

Fig. 2. Linear-temperature-programmed gas chromatograms of steam-volatile oil from nutmegs.

SUMMARY

Suitable conditions were developed for gas chromatographic separation of the components of cassia, black pepper, nutmeg, and ginger oils. Comparison of the peak heights of some of the major components of the spice oils showed marked and consistent differences between spices from different geographical origins. The characteristic components were identified by micro-infrared spectroscopy.

INTRODUCTION

Spices and spice oils contain complex mixtures of volatile aromatic flavoring compounds. Some of the volatile compounds of spices affect the olfactory centers. Since odor plus the effect on the taste buds determines what is called flavor, the flavoring characteristics of a spice oil are in part directly related to the nature and amount of its volatile components. The determination of geographical differences in the identities and amounts of components present would be of value in developing specifications for the spice industry. Early studies of the composition of spices have been comprehensively reviewed by Guenther (1952). These studies showed that among the identified components of spice oils are traces of low-boiling alcohols, esters, aldehydes, and ketones; large amounts of terpenes; and small quantities of many other less volatile compounds such as phenols or phenolic ethers.

Much effort has been directed toward characterizing the constituents of some spices and spice oils by chemical analysis. The work of Hasselstrom *et al.* (1957) is a good example of resolving a complex mixture of pepper oil and identifying its components by chemical analysis. A comprehensive study relating the composition of mint oils to geographical origin has recently been reported by Smith and Levi (1961). Jennings and Wrolstad (1961) reported a comprehensive study of components from commercial black pepper oil by gas chromatography and infrared spectroscopy. Early workers were handicapped because: 1) large quantities of spices or their volatile oils were required for complete chemical characterization; 2) laborious fractionation procedures had been necessary for separations and purifications; and 3) isolation of closely related and isomeric components was difficult if not technically impossible.

Since the introduction of gas-liquid partition chromatography, by James and Martin (1952), as an analytical method for the determination of volatile esters of fatty acids, this technique has vastly improved the possibilities of obtaining separation of complex volatile mixtures. The amount of a purified fraction obtained is frequently quite small. However, these isolated materials can be characterized by infrared microspectroscopy and mass spectrometry.

In conventional analysis of a spice lot, extensive characterizations are usually not undertaken. For example,

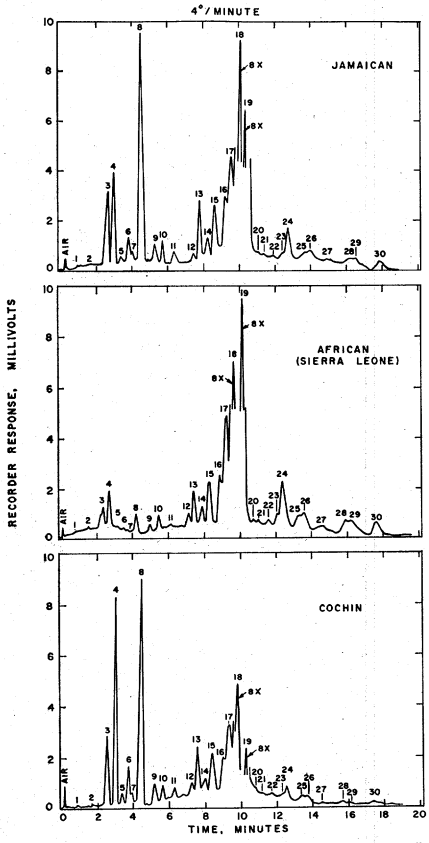

Fig. 3. Linear-temperature-programmed gas chromatograms of steam-volatile oil from gingers.

while the total percentage of steam-volatile oil in a spice sample is routinely determined (Clevenger, 1928; Lee and Ogg, 1956), the spice origin frequently cannot be verified by this type of analysis.

This paper describes the application of gas chromatography and infrared microspectroscopy to the identification of spice origins. The objectives were: 1) separation of the components of steam-volatile oils of some spices of different geographical origins; and 2) identification of a single typically different component or, in the absence of this, measurement of ratios between major components rather than an exhaustive qualitative and quantitative comparison.

METHODS

Materials. Authentic spice samples of different geographical origins were obtained from various reliable commercial spice companies by the American Spice Trade Association.

Apparatus. The gas chromatographic apparatus was assembled in this laboratory. A Research Specialty Company model 601–1 oven (no endorsement implied) served as the heating unit, a Gow-Mac two-filament thermal conductivity cell served as detector, and a constant-current voltage regulator served to control the heating of filaments. The injection port, detector block, column oven, gas inlet, and effluent outlet were kept at constant temperatures by employing separate controlling Variacs. Iron-constantan thermocouples were permanently attached to each component, and temperatures at the various locations were measured by turning a multiple-position switch connecting the thermocouples to a potentiometer. Helium was led to the inlet through a differential flowmeter to compensate for the pressure drop in the column. Stainless-steel tubes 6 ft long and ¼ in. OD were used as hairpin-type columns. Samples were introduced with a microliter syringe through a silicone-rubber septum. The recorder was a 10-mv full-span Leeds and Northrup type-G instrument with a one-second response and a chart speed of 0.5 in. per minute. This apparatus was used for separation and collection of the typically different fractions and the major fractions in which the ratio of the amounts of components differ.

Improved resolution and shorter analysis times for the separation of mixtures with a wide boiling range were achieved with linear temperature programming. An Aerograph model A-350-B gas chromatograph equipped with dual ¼-in.-OD × 5-ft stainless-steel columns, dual four-filament detector cell, and an automatic temperature programmer was used to obtain the linear programmed chromatograms shown in Figs. 1, 2, 3, and 4. The recorder was a Texas Instrument Servo-Riter with 1-mv sensitivity and 1-second pen speed.

Column preparation. In general, column packings were prepared by mixing a solution of liquid phase in a suitable solvent with Chromosorb-W by means of a magnetic stirrer. The packing mixtures were subsequently evaporated to dryness in a rotary vacuum evaporator at room temperature. A compressed-air vibrating tool aided uniform packing of the columns.

A large number of stationary phases were investigated to determine which would yield the best separation of spice oils into their component fractions. The stationary phases studied were Lac 446, Apiezon L, Dow Silicone 710, Hyprose SP 80, diethylene glycol succinate, Silicone grease, Sucrose-acetate isobutyrate (SAIB), Lac-3-R 728, di-n-decyl phthalate, butanediol succinate "Craig," diethylene glycol succinate (DEGS), Carbowax 400, 600, and 20M, and Ucon polar. The use of some of these sta-

Table 1. Gas-liquid chromatography operating conditions.[a]

Spice sample	Temp (°C) Column	Detector	Injector	Length and type of column	Liquid phase	Helium gas flow rate (ml/min)	Sample size (μl)	Chart speed (in./min)
Cassias								
Isothermal	185	250	250	6-ft. hairpin	Craig succinate (15%)	40	5	0.5
Linear program	89–220	300	300	5-ft. coil		50	2	0.2
Black Peppers								
Isothermal	195	250	250	6-ft. hairpin	Carbowax 20 M (15%)	40	5	0.5
Linear program	89–240	300	300	5-ft. coil		50	2	0.2
Nutmegs								
Isothermal	205	250	250	6-ft. hairpin	Carbowax 20 M (15%)	40	5	0.5
Linear program	89–240	300	300	5-ft. coil		50	2	0.2
Gingers								
Isothermal	210	250	250	6-ft. hairpin	Carbowax 20 M (15%)	40	5	0.5
Linear program	89–240	300	300	5-ft. coil		50	2	0.2

[a] The inert support in all columns was 60–80-mesh Chromosorb-W.

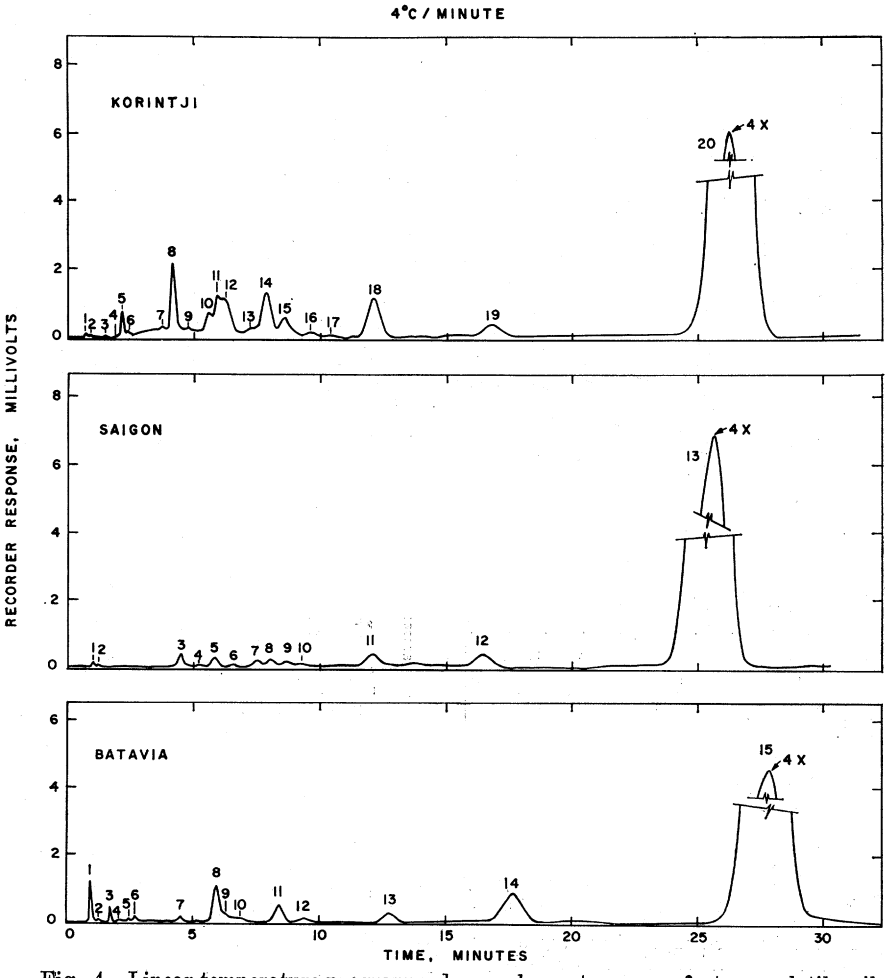

Fig. 4. Linear-temperature-programmed gas chromatograms of steam-volatile oils from cassias.

tionary phases in a two-column system was also explored.

Sample preparation. Three to ten samples of each spice from each geographical area were analyzed to determine if the differences between areas were consistent and greater than differences within an area. The samples were freshly ground, and the steam-volatile oil was obtained by the distillation procedure of Clevenger (1928) without the use of organic solvent. The freshly prepared oils were separated in an isothermal and a linear temperature programmed gas chromatograph. Table 1 shows the best conditions found for the separation of the components of each spice. The fractions isolated for infrared analysis were obtained under the conditions shown for the isothermal column.

A preliminary separation of hydrocarbon-enriched fractions and oxygenated-compound-enriched fractions of steam-volatile oils of spices on silicic acid columns were carried out by the method of Kirchner and Miller (1952). The method described by Stanley *et al.* (1961) was also used to separate the carbonyl-rich and carbonyl-poor fractions of these oils. The fractions obtained were examined by gas chromatography. The results showed that these enrichment procedures may be useful in comprehensive studies of volatile oil composition. These procedures were not used, however, because the objective of the present investigation was to establish if there were characteristic differences due to geographical origin rather than to make a comprehensive analysis of all components.

Collection of fractions and infrared spectroscopy. The typically different fraction and the major peaks in which the ratio of amounts of components differ were collected by a Model CH-6H fraction collector equipped with a type-"D" infrared absorption cell of 0.1 mm thickness, total volume of about 1 μl, manufactured by the Connecticut Instrument Company. After a fraction had been collected, the inner cell, 0.1 mm thick, was filled with CCl_4, and the solute was identified, where possible, by comparison of the infrared spectrum obtained by a Perkin-Elmer model 21 infrared spectrophotometer with spectra either from the literature or from authentic reference materials. When reference compounds were available they were purified by gas chromatography before their spectra were determined.

RESULTS AND DISCUSSION

The chromatograms discussed below were obtained on a limited number of samples from each source (3 to 10). Consequently, they do not indicate the limits of variability that might be encountered within samples from one area; nor do they give any indication of the year-to-year variation that may occur, for all samples were from one year's harvest. However, the data obtained indicate the usefulness of gas chromatography in identifying spice origin.

In all chromatograms, the notation 2×, 4×, 8× means that the amplification was reduced to ½, ¼, and ⅛ of its full value.

Fig. 1 shows chromatograms typical of black pepper oils from Lampong and Malabar. Malabar oil contained at least 31 volatile components, and Lampong oil at least 29. By superimposing the chromatograms of the two oils it was noted that Malabar peaks 18, 20, and 24 were not present in Lampong, whereas peak L of Lampong oil was absent in Malabar. Although peaks 18, 20, and 24 were present only in the chromatogram for Malabar oil, no attempt was made to identify them because they were not well resolved and were present in small amounts. These two oils differ in their relative concentrations of various fractions, particularly the major fractions 5, 6, and 16. In Lampong oil the ratio of fractions 5 and 6 was 1:05±0.1, whereas in the Malabar oil this ratio was 1:2± 0.2; the peak height of fraction 16 was always approximately twice as high in Lampong oil as in Malabar oil. Identification of the major fractions (5, 6, 16) was carried out in the hope of developing a simple chemical method of distinguishing these two black peppers. Table 2 presents the results of identification of these peaks by infrared spectroscopy.

Fig. 2 shows chromatograms typical of nutmeg oils from the East Indies and West Indies. Both nutmeg oils contain at least 25 volatile components. Typically different fractions other than possible trace components have not been noted. There were, however, differences in the relative amounts of major and minor components. These two nutmeg oils can be distinguished from each other by studying the ratio of fractions 1 and 3; in the East Indies nutmeg oils, the ratio between fractions 1 and 3 was 1:0.95 ± 0.05, whereas in the West Indies nutmeg the ratio between the same two fractions was 1:3.9 ± 0.1. Table 2 shows the identity of fractions 1, 3, 14, and 24 as determined by infrared spectroscopy.

Chromatograms typical of Jamaican, African (Sierra Leone), and Cochin ginger oils are shown in Fig. 3. All three ginger oils contain at least 30 volatile components. African ginger oil can be easily distinguished from Jamaican and Cochin ginger oils since the fractions 4 and 8 are absent (or present only as traces) in African ginger oil, and the ratio of the concentrations of fractions 18 and 19 is reversed. Jamaican ginger oil can be distinguished from that of Cochin by comparing the ratio of concentrations of fractions 4 and 8. The ratio between fractions 4 and 8 was 1:2 ± .05 for Jamaican ginger oil, and 1:0.95 ± .05 for Cochin ginger oil. The ratio of fractions 18 and 19 is calculated to be 1:0.7 ± 0.05 for Jamaican oils, 1:0.6 ± 0.05 for Cochin, and 1:1.4 ± 0.05 for African oil. The identity of the four peaks is shown in Table 2.

Fig. 4 shows chromatograms typical of cassia oils from Korintji, Saigon, and Batavia. These cassia oils respectively contain at least 20, 13, and 15 components. The lower-boiling components that may be characteristic of

Table 2. Identification of important components of spice volatile oils of different origins.

Fig. no.	Origin of spices	G.L.C. peak no.	Infrared identification
1. Black pepper	(Malabar, Lompong)	5	β-pinene
		6	D-limonene
		16	β-caryophyllene
2. Nutmegs	(East Indies, West Indies)	1	α-pinene
		3	Camphene
		14	d-terpineol
		24	Myristicin ?[a]
3. Gingers	[Jamaican, Cochin, and African (Sierra Leone)]	4	Camphene
		8	β-phellandrene
		18	Zingiberene
		19	Zingiberol
4. Cassias	(Saigon, Korintji, and Batavia)	1	Benzaldehyde
		last	Cinnamic aldehyde

[a] This material is an unsaturated ether without OH and C-O groups. It may be myristicin, but neither reference material nor infrared spectra were available for positive identification.

the origin of the oil either were present in trace amounts or were insufficiently resolved (component 11 in Korintji oil). It is evident from the three chromatograms that the origin of the cassia oils may be determined by gas chromatography because the relative concentration of some of the lower-boiling components and the number of trace constituents are different. Only the first and the last peaks obtained from these three oils were identified, as shown in Table 2.

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